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REVIEW OF THE RECENT LITERATURE ON THE HEALTH ASPECTS OF VITAMIN A, VITAMIN A ACETATE AND VITAMIN A PALMITATE AS FOOD INGREDIENTS

1977

Prepared for

BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
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Contract No. FDA 112-75-2004

by

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LIFE SCIENCES RESEARCH OFFICE
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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was prepared for the LSRO Select Committee on GRAS substances (SCOGS) as a part of their review of the health aspects of using these food ingredients as stipulated in the Food, Drug, and Cosmetic Act for Generally Recognized as Safe substances. Dr. Michael J. Wade prepared the report based on a comprehensive search and evaluative assessment of the current literature in accordance with the provisions of contract no. FDA 223-75-2004. Acknowledgement is made of the assistance of the LSRO staff who provided much of the background information.

Kenneth D. Fisher, Ph. D.
Director
Life Sciences Research Office

SUMMARY

This report reviews 91 recent publications from 1971 to 1976 concerning the health related effects of the use of vitamin A as a food ingredient. Topics include: the absorption, metabolism and storage of vitamin A in humans and animals; acute toxicity; short-term toxicity studies in five animal species; clinical reports of adverse effects of vitamin A exposure in humans; the protective and enhancing effects of vitamin A with respect to the actions of carcinogenic agents; and the teratogenic effects of vitamin A in mice and rats and speculations on its possible human teratogenicity. Additional topics discussed include the effects of vitamin A on membranes, epidermal tissues, lipid metabolism and the immune system.

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I. INTRODUCTION

This report concerns the health aspects of using vitamin A, vitamin A acetate and vitamin A palmitate as food additives. It reviews the world's scientific literature published from 1971 through 1976.

To assure completeness and currency as of the date of this report, information has been obtained by searches of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; and by the combined knowledge and experience of members of the LSRO staff. This report supplements and updates information contained in a scientific literature review (monograph) prepared for FDA by Informatics, Inc.[1]

Vitamin A (21 CFR 182.5930), vitamin A acetate (21 CFR 182.5933) and vitamin A palmitate (21 CFR 182.5936) are listed as generally recognized as safe in the Code of Federal Regulations[2] as nutrients and/or general purpose food additives. Vitamin A is also known as retinol (Figure 1). Vitamin A palmitate and vitamin A acetate are the palmitate and acetate esters of retinol. The term vitamin A is sometimes used when referring to esterified forms of retinol or to mixtures of retinol and its esters. There are a number of possible isomeric forms of retinol and retinyl esters arising from cis-trans isomerization about the double bonds. The most active form, and the one most frequently found in mammalian tissue, is the all-trans configuration; this is also the configuration of the commercial synthetic product. Although vitamin A is found exclusively in animal tissues, β-carotene, a substance found in plants, can be converted into vitamin A by most animal species.

In the literature, doses of vitamin A are most frequently expressed in micrograms or in terms of international units (IU); 1 μg of retinol is equivalent to 3.3 IU vitamin A.

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[1] The document is available from the National Technical Information Service. U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

[2] Office of the Federal Register, General Services Administration. 1977. Food and Drug Administration: rules and regulations. Food for human consumption. Reorganization and republication. Fed. Regist. 42: 14301-14469.

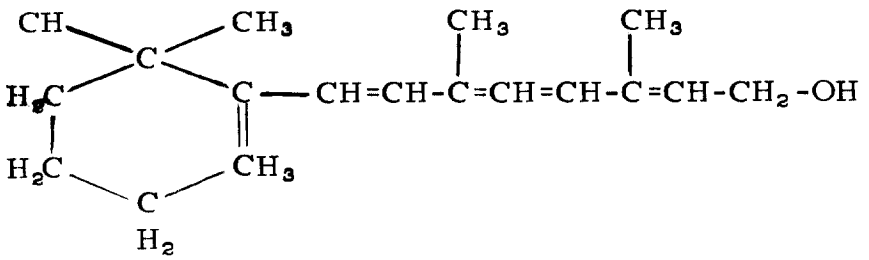

Figure 1. Structure of retinol.

II. ABSORPTION AND METABOLISM

In the intestine, retinyl esters are hydrolyzed to retinol which is absorbed by active transport, possibly with the involvement of a low-density lipoprotein. Absorption is enhanced by the presence of emulsifiers such as bile. Absorbed retinol is re-esterified, mainly to palmitate, and transported in the chylomicron fraction of lymph. It is taken up by the liver where it is stored in ester form. The liver stores account for over 90 percent of the total vitamin A in the body. A small amount of vitamin A may be absorbed in the stomach since 0.06 to 0.09 percent of an intubated dose of radioactive vitamin A was found in the livers of rats with ligated stomachs two hours after dosing (Kasper and Richter, 1971). Schneeberger *et al.* (1975) measured the serum level of vitamin A after oral administration of 150,000 IU (2500 IU per kg) to eight men. Serum vitamin A concentrations peaked at about 1320 IU (400 μg) per 100 ml 3 to 4 hours after dosing and fell to near normal values within 24 hours. There are several recent reviews of the literature on vitamin A absorption (Bauernfeind and Cort, 1974; Goodman, 1974; Mandel, 1975; Marks, 1975).

Retinol is conjugated to form a β-glucuronide which undergoes enterohepatic circulation and is oxidized to retinal and retinoic acid (Mandel, 1975). Retenoic acid is decarboxylated and further degraded; other metabolites are found in the urine and feces. Under ordinary conditions vitamin A does not appear in the urine.

The role of vitamin A in visual excitation has been elucidated by Wald (1968) and other workers (cf Fisher *et al.*, 1970). Considerably less is known about the biochemical details regarding the other physiological functions of vitamin A in maintaining fetal development, differentiation and integrity of epithelial tissues and fertility. The action of vitamin A on epithelial tissues was reviewed by Logan (1972), and De Luca and Wolf (1972).

Although some earlier studies identified the Kupffer cell as the site of liver vitamin A storage, work by Linder *et al.* (1971) implicated the hepatocyte as the storage site. Male Holtzman rats were fed diets containing either normal vitamin A levels (13 IU per g of chow) or excess vitamin A (1000 IU per g of chow) for 9 to 14 days. When the different cell types were isolated from livers of the rats, 96 percent of liver vitamin A was found in the hepatocytes and less than 4 percent in Kupffer cells at both vitamin A feeding levels. This same distribution was seen when radioactive vitamin A was measured in the liver cells of rats given ^{14}C vitamin A intravenously and sacrificed one hour later.

Kobayashi *et al.* (1973) injected 15 male Wistar rats with 25,000 IU of vitamin A in a castor oil vehicle twice a day for 6 days; in addition, there were vehicle-control and untreated control groups. The animals were killed 10 hours, 1 week or 6 weeks after the last injection. Light and electron microscopy of liver sections showed that the fat droplets in the "fat storing" cells of the vitamin A-injected animals were significantly larger and more numerous than in the vehicle control and untreated control animals. The largest increase in storage was in the rats killed 1 week after the last vitamin A injection.

Greatly enlarged hepatic sinusoidal cells containing numerous droplets were also observed by Tuchweber *et al.* (1976) in the livers of female Sprague-Dawley rats receiving 30,000 IU of vitamin A by stomach tube daily for 20 days. The hepatocytes of the vitamin A-treated animals were smaller than in untreated controls. These hepatic sinusoidal cells contained numerous droplets exhibiting an "evanescent fluorescence" characteristic of vitamin A.

Vitamin A stored in the liver is mobilized as retinol and transported to the tissues bound in a one-to-one complex with a retinol binding protein. This protein circulates as a dimeric complex with a pre-albumin protein. The metabolism and structure of retinol binding protein and pre-albumin were reviewed by Goodman (1974).

Studies with weanling, male Holtzman rats fed a vitamin A-deficient diet showed that as serum levels of vitamin A declined, serum levels of retinol binding protein also declined after a 3 day lag period (Muto *et al.*, 1972). There were appreciable amounts of apo-retinol binding protein formed in the livers of the vitamin A-deficient rats, presumably for secretion by the liver when additional vitamin A became available.

Smith *et al.* (1973) injected chylomicrons containing graded amounts of vitamin A into the tail veins of vitamin A-deficient male, weanling, Holtzman rats and observed a dose-related rise in serum retinol binding protein concentration after chylomicron clearance and a decreased concentration of retinol binding protein in the liver.

In man the liver vitamin A concentration is reported to be 100 μg per g of tissue and the plasma concentration to be about 30 to 70 μg per 100 ml (Mandel, 1975).

Sauberlich *et al.* (1974) used a radioisotopic method to measure the body pool of vitamin A in four individuals. The highest value of 877 mg was found in a 78 kg subject; the lowest value of 315 mg was found in a 59.5 kg subject and the heaviest subject (97.7 kg) had a pool of 412 mg of vitamin A.

Raica *et al.* (1972) analyzed liver and other tissue levels of vitamin A from human autopsy samples taken in five different states. Except for liver, the vitamin A concentration of most tissues was 1 μg per g or less. There was a wide range of liver vitamin levels from individual to individual. The overall mean liver concentration for 372 samples was 146 ± 151 μg per g of tissue with a median value of 106 μg per g. Twenty-two percent of the samples fell in the range of 0 to 40 μg per g; 17.2 percent had values between 200 and 500 μg per g; and 7.8 percent had more than 500 μg per g. There was no significant difference between vitamin A levels in samples from persons dying accidentally or from other causes; however there was a trend towards lower values in victims of accidental death. There were no significant differences in liver vitamin A levels between the sexes.

Mack *et al.* (1972) fed male, weanling rats (Sherman strain) a diet deficient in vitamin A until cessation of growth (110-120 g) occurred; after this time the rats received 8 μg of retinol acetate once weekly by mouth dropper until they reached a weight of 180-225 g. They were then injected with [11, 12-$^{3}H_2$] retinyl acetate and sacrificed 15 hours later. Liver and kidney plasma membranes were isolated and found to contain about 0.003 to 0.005 μg labeled retinol per mg of nitrogen. Liver and kidney homogenates had from 0.0014 to 0.009 μg of labeled retinol per mg of nitrogen, and red cell membranes contained about 0.0001 μg labeled retinol per mg of nitrogen. The radioactivity was strongly bound to the membranes and extraction of the membranes with chloroform-methanol indicated most of the radioactivity was associated with retinol and retinoic acid.

Leutskaya and Fais (1973) reported the presence of vitamin A in chicken and rat liver ribosomes even after treatment of the ribosomes with detergent.

Pereira and Begum (1973) fed 6 Indian children (age 4 to 5 years) 100,000 μg of retinol palmitate together with 14.4 μCi of [11,12-$^{3}H_2$] retinyl acetate as part of a meal. Serum retinol and radioactivity levels increased sharply 4 hours after dosing and declined over the following week. Fecal and urinary excretion of radioactivity, monitored for 8 days following administration, showed that the children absorbed from 68 to 80 percent and had retained 23 to 54 percent of the dose after the 8 days.

Rodriguez and Irwin (1972) extensively reviewed the literature on the vitamin A requirements of man, and Bauernfeind *et al.* (1974) reviewed recent investigations on vitamin A nutrition, giving extensive coverage to studies involving massive vitamin A dosing of populations whose normal diet is deficient in vitamin A. Single doses of up to 300,000 IU were given to children once or twice a year. These authors also reviewed studies on

the interactions of vitamin A and E, and suggested that vitamin A and E be given concurrently in massive dosing programs since vitamin E may be needed for efficient vitamin A utilization and storage, and in addition, may protect against hypervitaminosis A. High levels of vitamin A in the diet cause experimental animals to become deficient in vitamin E more rapidly.

Kusin *et al.* (1974) administered 200,000 IU of retinyl acetate to 17 normal children (weight, 10 to 15 kg). Concurrently 4 to 5 μCi of [11, 12-$^{3}H_{2}$] retinyl acetate and 0, 40, 100 or 500 mg of dl-α-tocopherol were given. Children receiving 40 or 100 mg of the tocopherol showed no additional absorption of the labeled retinyl acetate. The 500 mg dose of dl-α-tocopherol increased intestinal uptake of the labeled retinyl acetate but there was no significant effect on its retention.

III. ACUTE TOXICITY

McLaren *et al.* (1971) injected 20 adult Sprague-Dawley rats intraperitoneally with 200 to 500 mg of retinol per kg body weight. Plasma retinol and retinyl ester levels were measured 4 hours later. Only two of the animals survived; death occurred either within 40 hours or 3 days after injection. The retinol and retinyl ester levels were significantly lower (about 50 percent) at 4 hours in the animals that survived more than 40 hours. Similar results were obtained in weanling rats injected with 425 mg per kg retinol; survival time was inversely related to plasma retinol and retinyl ester levels.

IV. SHORT-TERM STUDIES

A. STUDIES WITH CATS

The effect on long bone growth of oral doses of 0.5 to 1.0 mg per kg per day of retinyl acetate for 21 or 30 days was studied in 10 newly weaned 6 to 8 week-old kittens (Clark, 1970). Pair-fed controls were utilized to compensate for any change in food intake caused by vitamin A dosing. Eight of the 10 pairs of kittens were sacrificed after the dosing period, while two pairs were put on normal diets for some[1] weeks before being sacrificed. Average daily food intake of the animals was depressed by 20 to 80 percent of their starting levels. Three of the test animals lost weight during dosing while all 10 controls were healthy and gained weight during the experiment. By contrast, the dosed group had dull coats and were reluctant to walk during treatment. The kittens put on a normal diet at the end of dosing gained weight and became healthier; however, they developed an abnormality of gait. At necropsy the dosed kittens weighed less than their controls, were dehydrated and had less subcutaneous fat. Bones of the dosed animals were more brittle than the controls; the long bones were shorter and narrower in shaft diameter and the epiphyses were proportionally reduced. There was damage to many epiphyseal growth plates and marked osteoporosis of the diaphyses. Levels of vitamin A in the livers and kidneys of the dosed animals were greatly increased.

B. STUDIES WITH DOGS

Cho *et al.* (1975) studied the toxic effects of vitamin A on 3 groups of Labrador retriever pups; each group consisted of 2 animals. Group II received weekly intramuscular injections of retinyl palmitate (100,000 IU per kg) for 10 weeks, then were given daily oral doses of 300,000 IU per kg for 88 days. Group III received 200,000 IU per kg of retinyl palmitate injected intramuscularly once a week for 14 weeks. Group I was the control group, but after 72 days one of the animals received 300,000 IU of retinyl palmitate per kg of body weight daily for 48 days while the remaining control animal received 4 ml of corn oil daily. At the 11th week, pups in group II and III were dehydrated and had central nervous system depression. After 14 weeks, group II pups were severely dehydrated, emaciated, reluctant to walk, and exhibited signs of depression. The dog transferred out of the control group on day 72, was the most severely

[1] Number of weeks not specified.

affected and died on day 121 (day 48 of vitamin A dosing). During the treatment period hemogram values were within normal limits except for slight decreases in hemoglobin, packed red cell volume and serum calcium, and increases in organic phosphorus and alkaline phosphatase in the group II animals. Radiologic examination showed early epiphysitis of the long bones on day 50 and reduced cortexes and epiphyseal plates of the long bones and thinning and demineralization of the fibulas by day 150 in the group II dogs; some radiologic changes were also noted in group III animals. No gross changes were detected at necropsy except fatty liver and those previously seen on radiographic examination. Histopathologic examination showed dilation and microcalculi in degenerative distal and collecting renal tubules of group II animals. Sections of liver from group II and III animals had dilated central veins, cloudy swelling and fatty changes of hepatocytes and a few random foci of mononuclear cells. Sinusoids were collapsed because of swollen hepatocytes which were of "clear ground glass appearance" and contained empty, round vacuoles in the cytoplasm. Fatty changes were diffuse and of moderate grade with some tendency towards centrilobular distribution.

C. STUDIES WITH CALVES

Ten-week-old Holstein calves in groups of five to seven animals were fed rations depleted in vitamin A and then supplemented with retinol acetate to provide the equivalent of 54, 108, 8,800 or 17,600 µg of retinol per kg body weight per day (Gorgacz *et al.*, 1971). The animals remained on the diets for 12 weeks. The 54 and 108 µg per kg levels were considered control intakes while the 8,800 and 17,600 µg per kg levels were considered moderately and severely toxic, respectively. The animals receiving the two highest levels of vitamin A consumed significantly less feed than the control groups and the 17,600 µg group ate significantly less than the 8,800 µg group. The hypervitaminotic groups also showed significantly less weight gain and had a shorter height at the withers than the control groups. At the end of the experiment the heart rates were 99, 97, 120 and 136 in order of increasing vitamin A intake. The hypervitaminotic A groups had characteristic signs of vitamin A toxicity including hyperhydrosis, alopecia and erythema, hyperemia of the oral, nasal and anal mucosae, lameness, abnormal stance and abnormal horn growth. Average plasma and liver vitamin A concentrations increased with increasing vitamin A dosage. As compared to control groups the two high dosage groups had significant increases in brain and kidney weight when expressed on a per unit live weight basis. The 17,600 µg vitamin A group had significantly higher adrenal gland weight. Dry matter content of the tentorium cerebelli portion of the dura matter was significantly less in the hypervitaminotic A calves as were total mucopolysaccharides, particularly the chondroitin sulfate fraction.

In a similar experiment Gorgacz *et al.* (1975) fed groups of Holstein-Frieser male calves diets containing 108, 8,800 or 17,600 µg per kg per day of retinol equivalents of retinyl acetate for 12 weeks. The arachnoid granulations were reduced in size and the choroid plexus epithelial cells were reduced in height in the 8,800 and 17,600 µg per kg groups.

D. STUDIES WITH RABBITS

Akinosho and Basu (1971) injected 6 albino rabbits intraperitoneally with one million units of retinyl acetate daily for 10 days. These animals and 3 controls all weighed about 1 kg at the beginning of the experiment. The control rabbits all gained weight during the study while the experimental group lost an average of 0.34 kg. All the experimental animals showed bilateral ear collapse. Clinically, the eyes of the experimental and control animals were similar, but eyes of some experimental animals showed slight exudates and conjunctival dryness. However, upon histologic examination significant differences were apparent with reduction of the corneal mucoid and reduced cell number and changed morphology of the goblet cells of the conjunctiva in the vitamin A-treated group.

E. STUDIES WITH RATS

Mallia *et al.* (1975) performed two studies on hypervitaminosis A in the rat. In study I weanling male rats (Holtzman strain, 51 to 63 g body weight) were assigned to 3 groups of 10 animals each: group 1 (control) received 0.14 mg of vitamin A daily (2.5 mg per kg); group 2 received 7.3 mg of retinyl esters daily (133 mg per kg); and group 3 was given 1.8 mg of retinyl esters daily (33 mg per kg) until day 25 when they were given 41 mg of retinyl esters daily (745 mg per kg). After day 50 groups 2 and 3 received no vitamin A; group 1 continued to receive 0.14 mg daily until the end of the experiment on day 99. The growth rate of all three groups was comparable until day 26. At this time the rats in group 2 showed a slightly lower but insignificant reduction in growth rate; this group remained healthy throughout the experiment. When the dose of vitamin A was increased for the group 3 rats at day 25, they ceased to grow and exhibited other signs of hypervitaminosis A including reduced food intake, alopecia of the head, thickening of the skin, occasional bleeding from the nose and weakness or partial paralysis of the legs. Unlike the controls, they were aggressive rather than docile when handled. Upon discontinuation of dosing on day 50, the group 3 rats slowly recovered and then continued to grow until the end of the study. The total plasma vitamin A level of group 1 animals increased slightly to about 65 to 70 µg

per 100 ml during the first 2 to 3 weeks of the study then declined to a plateau value of about 40 to 60 μg per 100 ml. In the group 2 animals the total plasma vitamin A level rose to 103 μg per 100 ml by day 16 and then slowly declined. Similar results were seen in the group 3 animals with plasma levels rising to 102 μg per 100 ml before declining slowly. Levels of retinol binding protein, retinol and retinyl esters were determined separately in liver and plasma. The levels of retinol were comparable in control and hypervitaminotic animals at all times, at a value of around 30 to 50 μg per 100 ml. Less than 10 percent of the total plasma vitamin A was present as retinyl esters in the control group; however, when total plasma vitamin A levels were elevated in the group 2 and 3 animals; the percentage of vitamin A as retinyl esters was markedly elevated by 40 percent or higher. At day 50 of the experiment, groups 2 and 3 had about a 25-fold increase in liver vitamin A content as compared to controls. Since both of these groups had a similar vitamin A content despite the higher dose received by group 3, the investigators suggested that the liver has a finite storage capacity for vitamin A. The animals in groups 2 and 3 developed fatty livers with about twice the total liver fat as the control animals. Plasma levels of retinol binding protein remained fairly steady throughout the experiment. There was some fluctuation in the levels observed in groups 2 and 3 but they generally remained lower than the control values. The liver levels of retinol binding protein were similar for groups 1 and 2 and decreased by one-half in the livers of the group 3 animals.

In the second study performed by Mallia *et al.* (1975), the control group received the same treatment as described in study I and the hypervitaminotic A group received 34.4 mg daily of retinyl acetate (625 mg per kg). There were 16 rats in each group and the study lasted 23 days. The hypervitaminotic group also lost weight (a mean of 10 g in 22 days) and had signs of vitamin A toxicity. Both groups received tracer amounts of ^{3}H retinyl acetate daily. On day 23 of the experiment, samples of blood from both groups were fractionated by ultracentrifugation to obtain lipoprotein and nonlipoprotein fractions. Aliquots of each of these fractions were separated further to obtain estimations of retinol and retinyl ester content. In the control rats only 18 percent of the vitamin A was found in the lipoprotein fraction and 82 percent in the nonlipoprotein fraction. By contrast the hypervitaminotic group had 84 percent of the vitamin A in the lipoprotein fraction and 16 percent in the nonlipoprotein fraction. In the hypervitaminotic group 88 percent of the vitamin A associated with the lipoprotein fraction was in the form of retinyl esters while in the control group only 3 percent of the vitamin A in the nonlipoprotein fraction was in the form of retinyl esters.

Mallia *et al.* (1975) concluded from the results of both studies that serum lipoproteins play an important role in the transport of vitamin A

during hypervitaminosis and that toxic manifestations of vitamin A overdosing appear when vitamin A circulates in the plasma in a form not bound to retinol binding protein. These authors speculated that plasma lipoproteins may nonspecifically deliver vitamin A to biological membranes and thus lead to toxicity.

V. OTHER STUDIES

A. CLINICAL STUDIES

Siegel and Spackman (1972) described a case of two young siblings who developed hypervitaminosis A as a result of chronic administration of vitamin A supplement by their mother. The 30-month-old boy was admitted to the hospital with anorexia, lethargy and an inability to walk because of pain in both shins. He had an enlarged head, slightly enlarged liver and spleen, and alopecia. He had been receiving at least 57,000 IU of vitamin A daily for about a year and had a (markedly increased) serum vitamin A level of 520 μg per 100 ml (normal 30-80). His 12-month-old sister, also hospitalized with vomiting and irritability, had been taking about 25,000 IU of vitamin A daily for 9 months. She had exfolitive dermatitis, a palpable liver, a bulging anterior fontanel and a serum vitamin A level of 180 μg per 100 ml. Radiographic examination revealed a pronounced widening of the cranial sutures. After discontinuation of the vitamin A supplementation, both children showed rapid amelioration of symptoms including normal skull x-rays within two months.

Symptoms of prolonged depression, insomnia and poor appetite developed in an 18-year-old female 6 months after she began taking 50,000 IU of vitamin A two or three times daily for acne (Restak, 1972). The patient was admitted to the hospital 20 months after beginning vitamin A therapy with a 7-week history of frontal headaches, sleep disturbances, blurred vision and tinnitus. Physical examination was normal except for bilateral papilledema. Vitamin A dosing was stopped and a preliminary diagnosis of intracranial neoplasm was made. Radiographic, electroencephalographic and metabolic studies were all normal except for 50 μg per 100 ml of serum vitamin A found 1 week after discontinuation of vitamin A. Two weeks after admission the patient's symptoms, including papilledema, began to resolve with total recovery in 6 months.

Bilateral papilledema was also seen in an 18-year-old male who had taken 10,000 to 20,000 IU of vitamin A daily for more than 2 years and complained of difficulty with equilibrium and a tendency to lean to one side. Computerized axial tomography showed a very small third ventricle and small lateral ventricles, presumably a result of benign intracranial hypertension due to vitamin A overdose; the patient became symptom free upon withdrawal of the vitamin. (Vollbracht and Gilroy, 1976).

Another instance of papilledema in conjunction with chronic vitamin A intoxication was seen in a 15-year-old girl who had been taking 200,000 IU of vitamin A daily for 2 years for acne treatment (Hawkins and Burlon, 1974).

Aucte renal failure occurred in a 22-year-old man who had taken 70-75 million IU of vitamin A over a 38-day period as a treatment for psoriasis (Földi *et al.*, 1976). The treatment was stopped because of signs of vitamin A poisoning; acute renal failure occurred 9 days later. Seven hemodialyses were performed and the patient recovered within 12 days. A liver biopsy showed normal histology, however renal biopsy indicated acute necrosis of the renal tubules had occurred.

Frame *et al.* (1974) described three patients with hypercalcemia and skeletal pains who had ingested 75,000 IU or more of vitamin A daily for several years. However, these patients were also taking vitamin D supplements which may have contributed to their symptoms.

Smith and Goodman (1976) found that in three patients admitted for chronic vitamin A poisoning, retinyl esters accounted for 41 to 67 percent of the total serum vitamin A even when measured up to 3 weeks after discontinuation of vitamin A dosing. By contrast, in 14 normal patients retinyl esters accounted for 0.1 to 4.7 percent (mean 1.6 percent) of the total serum vitamin A. There was a molar excess of vitamin A over retinol binding protein in the poisoned patient's serum leading these workers to propose that vitamin A poisoning occurs when excessive amounts of vitamin A are presented to cell surfaces in association with plasma lipoproteins rather than retinol binding protein.

Hypercalcemia occurred in an 18-year-old woman taking 150,000 IU of vitamin A daily for 3 years (Fisher and Skillern, 1974) and in a 16-year-old boy taking 100,000 to 300,000 IU daily for 6 months (Wieland *et al.*, 1971). On admission these patients had serum calcium levels of 15 mg per 100 ml and 18.9 mg per 100 ml respectively.

A review of 517 cases of acute vitamin A intoxication and chronic hypervitaminosis A was made by Körner and Völlm (1975); and the American Academy of Pediatrics Committees on Drugs and Nutrition, (1971) issued a joint statement on the use and abuse of vitamin A which points out the possible hazard of overdose by the use of high potency over-the-counter vitamin A preparations.

B. TERATOGENIC EFFECTS

In 1953 Cohlan associated high doses of vitamin A in pregnant Wistar rats with defects in their offspring; since then, there have been numerous accounts of its teratogenicity (cf. Informatics, 1974). Animal studies have shown an association between high doses of vitamin A given to pregnant animals and abnormalities such as hydrocephalus, encephalocele, cleft palate and other abnormalities including defects in development and learning even in the absence of structural abnormalities (Hutchings and Gaston, 1973).

Abramovich (1973) injected groups of eight pregnant rats with 100,000 IU of vitamin A each, either on the 10th day of gestation or the 10th and 11th days. The fetuses were obtained on the 18th day and fixed and sectioned. Fetuses were also obtained from control animals not dosed with vitamin A. Almost all the vitamin A exposed fetuses had cleft palate shelves and other abnormalities of the facial structure. Compared to control fetuses, the vitamin A exposed group had a significant decrease in the height of the nasal septum cartilage and a significant increase in the thickness of the mandible.

Nanda (1970) also observed cleft palate in embryos from 14 pregnant Wistar rats dosed intragastrically with 400,000 IU of retinyl palmitate on days 9 through 12 of gestation. The embryos were recovered on day 19. Of 121 implantation sites, 12 were resorbed and the remaining 109 embryos had cleft palate, and some showed other anomalies such as syndactyly, shortened fore and hind limbs, anomalies of the eyes and ears, and exencephalia. Some embryos were fixed and sectioned for histological examination, and abnormalities such as defects of the salivary glands, absence or abnormality of the maxillary and mandibular tooth buds and absence of components of the temporomandibular joint were found. No abnormalities were found in control fetuses not exposed to vitamin A.

Nakamura (1975) cultured forelimb buds of embryos taken from ICR-JCL mice on the 11th day of pregnancy. Forelimb buds cultured in the presence of 10 IU per ml of retinol showed decreased chondrification of metacarpals and inhibition of keratinization as compared to control explants cultured in the absence of retinol. Uptake of radiolabeled sulfate was also decreased in the retinol-treated explants. The investigator concluded that retinol acts directly on limb buds and supresses formation of chondroitin sulfate and inhibits keratinization.

Terashima and Nogami (1974) studied cartilage metabolism in fetal Sprague-Dawley rats whose mothers received an intravenous injection of 100,000 IU of vitamin A on the 11th day of pregnancy. Carrier free ^{35}S-sulfate was injected into the vitamin A treated and the control mothers

on days 15, 16 and 18 of pregnancy. The results of pulse-chase experiments with a 1-hour labeling period indicated vitamin A exposure increased both synthesis and degradation of sulfated glycosaminoglycans on day 15 of pregnancy. These workers speculated that the accelerated degradation rate may have been due to activation of lysosomal enzymes by vitamin A. Total incorporation of ^{35}S-sulfate into cartilage glycosaminoglycans of the extremities was greater on days 15 and 16 in the vitamin A exposed animals and was markedly reduced in 18- and 22-day fetuses (22-day fetuses are newborns). The molecular weight distribution of the glycosaminoglycan fraction of cartilage, as measured by gel filtration, appeared similar in samples taken from newborn control and vitamin A treated animals.

Morriss (1972) made a detailed morphological examination of abnormalities occurring in fetal Wistar rats obtained at days 9, 10 and 11 from pregnant animals injected with 100,000 IU of vitamin A palmitate on the 8th day of gestation. He suggested the malformations originated from a loss of synchrony in the development process because of a differential toxicity of vitamin A to the three germ layers.

Retinol had a direct teratogenic effect on rat embryos explanted on the 8th day of gestation and cultured in the presence of 0.5 to 20 µg per ml retinol for 48 hours (Morriss and Steele, 1974). Compared to controls, very poor development occurred in embryos cultured in media containing 5, 10 or 20 µg per ml retinol; none had a beating heart and little or no differentiation had occurred. These workers stated that after allowance for differences due to development *in vitro* the morphology of embryos cultured in 0.5 µg retinol per ml for 48 hours was strikingly similar to embryos explanted 48 hours after maternal administration of 60,000 µg of vitamin A, suggesting that the compound has a direct teratogenic effect on embryonic development.

The presence of 5 or 10 IU of retinol per ml of medium caused destruction of the extracellular matrix of cultured explants of chick limb bone rudiments; when the same amount of retinol was complexed with retinol binding protein and added to the culture medium, no destruction was noted (Dingle *et al.*, 1972).

Recent studies carried out *in vitro* show that vitamin A at a concentration of 10 IU per ml may interfere with cell movement in cultured mouse limb buds (Kwasigroch and Kochhar, 1975), and cell division and metabolism in cultured embryonic chick sterna (Vasan and Lash, 1975).

Solursh and Meier (1973) cultured embryonic cartilage cells from the sterna of 13-day-old chick embryos and found that incubation with 0.003 to 30 µg per ml of vitamin A for 24 hours caused a dose-dependent inhibition of acid mucopolysaccharide synthesis although collagen synthesis was not inhibited.

The behavioral effects of maternal hypervitaminosis A were investigated in the offspring of pregnant Fischer rats administered 10,000, 25,000, 40,000 or 100,000 IU of retinyl palmitate per kg on days 8, 9, and 10 of gestation (Vorhees, 1974). At 70 days of age there were only five surviving offspring from eight litters of mice at the 100,000 IU per kg dose level. At the three lower doses there was no mortality and, compared to untreated controls, no differences in body weight among the offspring at age 70 days. However, when tested at age 70 to 72 days all of the vitamin A-exposed animals showed impairment in learning behavior as measured by a maze avoidance test.

Hutchings and Gaston (1974) administered 90,000 IU of vitamin A intragastrically to pregnant rats on days 17 and 18 of gestation. The offspring had significantly reduced learning scores as compared to vehicle-treated and untreated controls when tested by lever training to receive a water reward or for auditory discrimination. Vitamin A treatment had no effect on the brain weight or dimension(s) of the offspring.

Butcher *et al.* (1972) also reported significant impairment in maze learning performance in the offspring of Sprague-Dawley rats receiving 100,000 IU of vitamin A per kg of body weight by stomach tube on the 8th, 9th and 10th day of pregnancy.

Gal *et al.* (1972) found a higher level of vitamin A in blood obtained 7 days postpartum from women delivering babies with central nervous system defects than in blood samples obtained from women delivering normal babies. The investigators, however, were unclear about the significance of their results since there is a wide variation in blood vitamin A levels, and no information was available on serum vitamin A levels during pregnancy.

Women taking oral contraceptives have higher levels of plasma vitamin A but two recent reviews concluded that this probably represents no risk to the offspring of women taking oral contraceptives shortly before becoming pregnant (Wild *et al.*, 1974 and Larsson-Cohn, 1975).

C. LACTATION STUDIES

Lopes *et al.* (1974) studied testicular development in 90 young male Wistar rats whose mothers received daily intraperitoneal injections of 40,000 IU of retinyl palmitate during lactation. Animals were sacrificed at ages 1, 5, 10, 14, 20 and 25 days and histological analyses of the testes of treated rats were compared with those of control rats of similar age whose mothers received daily saline injections. No differences between

the two groups were seen at ages 1 and 5 days. However, in the older groups whose mothers received vitamin A there was a decrease in the number of mitoses and a delay in development of seminiferous tubules.

D. EFFECTS OF VITAMIN A ON MEMBRANES

Wasserman and Corradino (1971) reviewed some aspects of the interaction of vitamin A with membranes. Vitamin A can interact with lysosomal membranes *in vivo* and *in vitro* to cause release of lysosomal enzymes. Dewar *et al.* (1975) found that a 45-minute incubation of lysosomes isolated from rat retinas with 1.0 to 2.5 μg of retinol per mg wet weight of retina caused an increased release of β-glucuronidase, β-galactosidase and hexosamidase from the lysosomes. Similarly, Wang *et al.* (1976) found that retinol and retinyl acetate caused the release of acid phosphatase, β-glucuronidase, deoxyribonuclease and N-acetyl-β-D-glucosaminidase when incubated *in vitro* with lysosomes from mouse liver. Retinol caused a greater release of the enzymes than retinyl acetate.

Extensive hemolysis results when human red cells are exposed for 20 minutes to 40 μg per ml of retinol (Murphy, 1973). Scanning electron microscopy of the exposed cells showed that even at 1-minute exposures the cells are swollen with localized unfoldings of the plasmalemma. Another of the first effects noted was the transition from the typical biconcave disc to cells possessing one enlarged concavity with small invaginations present within the concavity.

E. EFFECTS OF VITAMIN A ON THE IMMUNE SYSTEM

There are some reports in the literature suggesting that vitamin A dosing can affect the function of the immune system. Cohen and Cohen (1973) injected 7- to 12-week-old female C57BL16J and CBA male mice (18 to 24 g) intraperitoneally for four consecutive days with doses up to 9,000 IU of retinyl palmitate (500,000 IU per kg, assuming 18 g mice). On the day of the last injection the mice were immunized with sheep red blood cells and four days later spleen cell suspensions from the mice were prepared and the number of 19S antisheep red blood cell antibody-forming units per spleen cell were measured. Administration of less than 1,000 IU of vitamin A per day (56,000 IU per kg) caused no change from the normal antisheep red blood cell response; however the number of antibody forming units increased significantly in mice receiving 1,000 IU vitamin A per day, the peak

response, about a sixfold increase over control values, was seen in mice receiving 3,000 IU per day (167,000 IU per kg). Animals receiving 9,000 IU of vitamin A had only a twofold increase over control values, and in addition showed signs of toxicity such as weight loss, ruffled fur and hypoactivity. Vitamin A dosing also increased the antibody response of the mice to immunization with a dinitrophenol-ovalbumin conjugate.

Intraperitoneal injection of 35,000 IU retinyl palmitate (70,000 IU per kg) daily for three months to a group of 10 male guinea pigs caused lymphomonocytosis and marked changes in organs of the lymphomyeloid complex (Polliack and Drexler, 1972). In vitamin A-treated animals, absolute lymphocytosis and monocytosis of the peripheral blood were evident. Myelograms of the treated animals showed reductions in the granulocyte series and increased numbers of lymphocytes and monocytes. Changes in the spleens of the animals included frequent hyperplasia of the white pulp and marked proliferation of lymphocytes in the red pulp. Extensive changes occurred in the lymph nodes including replacement of the architecture with sheets of lymphocytes. The presence of many cells with phagocytic activity was noted in the bone marrow, and the vitamin A treated animals showed an increased incorporation of tritiated thymidine into DNA of spleen lymph nodes and bone marrow.

F. EFFECTS OF VITAMIN A ON CEREBROSPINAL FLUID

Maddux *et al.* (1974) investigated the effect of vitamin A on intracranial pressure and brain water in rats. The animals were administered retinol through a catheter inserted into the esophagus. Male Sprague-Dawley rats, both immature (45 to 125 g) and mature (180 to 400 g), were used. A group designated as the "acute group" received one dose of 15 mg (immature) or 30 mg (mature) of retinol. In the group designated "chronic" the immature rats received 7.5 mg of retinol per day for three to eight days and the mature animals received 15 mg per day for 7 or 8 days. No change in cerebrospinal fluid pressure was seen in rats from the "acute group". However, as compared to control animals the "chronic group" showed a 73 percent drop in cerebrospinal fluid pressure after 3 to 5 days of dosing and a 93 percent decrease after 6 to 8 days of dosing. The integrity of the system providing resistance to cerebrospinal fluid outflow was investigated by measuring the rate of pressure change in control and chronically-dosed animals when the cerebrospinal fluid pressure in the chronic group was raised to the same level as the control group. The chronically-dosed rats showed a drop in cerebrospinal fluid pressure within 4 minutes after it was adjusted to normal pressure, indicating a loss of resistance to outflow in these animals. Brain volume increased by 2.0 percent in the chronically-treated immature rats and 4.8 percent in the mature chronic group while no changes in brain volume were found in the "acute group" of vitamin A treated animals.

G. EFFECTS OF VITAMIN A ON EPIDERMAL TISSUES

Sweeny and Hardy (1975) found that keratinization of skin from the upper lip of 12-day-old embryonic Swiss mice was suppressed when cultured for 10 days in the presence of 5.7 μg per ml retinol; a ciliated and secretory epithelium developed in these cultures. By contrast, squamous stratification and keratinization occurred in skin cultures to which retinol was not added.

Addition of 1.56 or 3.12 μg per ml of retinyl acetate to BALB/c mouse epidermal cells cultured in a defined medium resulted in an increase of 40 percent in the cellular RNA content (Sporn *et al.*, 1973). Treatment with more (6.25 μg per ml) or less (0.78 μg per ml) retinyl acetate had a lesser effect on cellular RNA content.

H. EFFECTS OF VITAMIN A ON LIPID METABOLISM

There are a number of recent studies by Indian workers concerning the effects of high doses of vitamin A on lipid metabolism in the rat. Misra (1974) fed groups of 6 Wistar male rats doses of 0, 4.2, 16.9 or 27.5 mg of retinol (26, 105, and 172 mg per kg) daily for 10 days. The rats fed the highest dose lost a significant amount of weight during the 10 days. There were significant increases in liver total lipids, triglycerides and esterified cholesterol at all three retinol feeding levels as well as significant increases in total fatty acids at the two highest feeding groups, and a significant increase in total cholesterol at the highest feeding group. On the 10th day of the experiment the rats were injected with ^{14}C sodium acetate, and there was a significant increase in incorporation of label into hepatic-free cholesterol in all feeding groups and into hepatic-total cholesterol in the highest retinol feeding group. The ^{14}C specific activity of hepatic triglyceride was significantly lower in all retinol-fed rats. The total liver-phospholipid phosphorus concentration was not affected by retinol feeding, although variations between feeding groups were noted in some of the individual phospholipid compounds.

Ahuja and Misra (1975) fed groups of 12 male Wistar rats (90 to 100 g weight) 0 or 33 mg of retinol per day for 2 days (330 mg per kg). One day after administration of the last dose the rats were injected with 1-^{14}C palmitic acid through the tail vein and sacrificed 10 to 60 minutes later. The liver weight of the rats on a mg per 100 g body weight basis was higher in the retinol-fed rats. They also had significantly higher concentrations of liver triglyceride, phosphatidylcholine, phosphatidylethanolamine and

plasma-free fatty acids than the control animals, and the incorporation of labeled palmitate into liver triglyceride, phosphatidylcholine and phosphatidylethanolamine was higher in the retinol-fed rats.

Misra and Srivastava (1974) reported that feeding of 50,000 IU of vitamin A (chemical form not stated) daily for 2 days to six female Wistar rats (500,000 IU per kg) lowered the adrenal levels of cholesterol and ascorbate as compared to controls fed only the vehicle.

Ramachandran *et al.* (1974) found about twofold higher levels of plasma-free fatty acids and mobilized-free fatty acids in the epididymal fat pads of six male Wistar rats fed 30,000 IU of retinol per day (375,000 IU per kg) for 2 days. These workers also reported higher levels of total lipid and triglycerides in the livers of the dosed rats as compared to controls.

I. SENSITIZATION TO VITAMIN A

An allergic reaction developed in a 53-year-old female about 3 hours after taking vitamin A capsules prescribed by her physician for dry skin (Palei, 1971). Although this was the patient's first exposure to a vitamin A preparation, she had a history of allergic reactions to strawberries. The patient's signs and symptoms included facial edema, pruritis of the skin, headache, difficult breathing, cardiac palpitations and bronchial edema.

J. STUDIES ON ATHEROSCLEROSIS AND VITAMIN A

Ten New Zealand white male rabbits fed a diet containing 0.2 percent cholesterol and vitamin A acetate (25 million IU per 100 g of diet) for one year had somewhat reduced hypercholesterolemia and less severe atherosclerotic lesions as compared to a group of 10 rabbits receiving 0.2 percent cholesterol but no supplemental vitamin A in the diet (Bonner *et al.*, 1973). High dietary levels of vitamin A have also been reported to reduce the severity of atherosclerosis in Japanese quail (Bayer *et al.*, 1972), while divergent effects in different strains were found in chickens (Woodward and March, 1974).

K. VITAMIN A AND CANCER

Maugh (1974) briefly summarized reports suggesting vitamin A may have the potential to inhibit the carcinogenic response to a viral or chemical carcinogen during the pre-neoplastic phase after initiation of the response but prior to cellular transformation. Clayson (1975) recently published a brief review of the protective and enhancing effects of vitamin A on carcinogenesis, and concluded that only further critical experimentation can clarify the present confused situation regarding vitamin A and carcinogenesis. Sporn *et al.* (1976) concluded there is good evidence supporting the thesis that retinoid deficiency is linked to an increased risk of cancer from chemical carcinogens. In addition, these reviewers concluded that under some conditions natural retinoids can prevent the development of epithelial cancer, but are limited in their usefulness as chemopreventive agents either because of inadequate tissue distribution or their toxicity.

Felix *et al.* (1975) found that only 17 percent (4 out of 24) of a group of BALB/c mice who received 3100 IU of retinyl palmitate daily in their drinking water developed tumors after challenge with an injection containing a suspension of cells of a transplantable murine melanoma. The animals received the injections 2 weeks after addition of retinyl palmitate to their drinking water and continued on the vitamin until 2 weeks after the first tumors appeared. All 25 of the unsupplemented control animals developed tumors.

A lower rate of tumor growth, but not of tumor incidence, was found in three groups of C3H/HeJ female mice (10 or 20 mice per group) inoculated with C3HBA tumor cells and receiving 150,000 IU of retinyl palmitate per kg of diet as compared to control animals inoculated with the tumor cells but not receiving supplemental vitamin A (Rettura *et al.*, 1975). The control animals had a mean survival time of about 43 days after inoculation while the survival times of the experimental groups were 63, 75 and 54 days, respectively.

Smith *et al.* (1975a; 1975b) found that groups of 52 to 57 male, Syrian golden hamsters housed in laminar flow cages and receiving 1600 or 2400 μg of retinyl acetate per week intragastrically had a somewhat lower but statistically insignificant incidence of respiratory tumors induced by benzpyrene compared to laminar flow-housed controls receiving 100 μg of retinyl acetate weekly. By contrast, when the same experiment was repeated with

conventionally-housed animals the two groups receiving high doses of retinyl acetate had a higher incidence of respiratory tract tumors. Regardless of housing conditions, there was a significant reduction of squamous papillomas of the forestomach in animals receiving high doses of vitamin A.

L. OTHER MISCELLANEOUS EFFECTS OF VITAMIN A

Woodward and March (1974) found an increase in prothrombin times in 10 Black Australorp chicks fed 100,000 or 200,000 IU of retinyl palmitate per kg per day for 3 weeks; no effect was noted at the level of 5000 or 35,000 IU per kg. Similar treatment did not affect prothrombin times in White Leghorn or New Hampshire chicks.

Changes in glycosaminoglycan metabolism were found in a group of 30 young male Sprague-Dawley rats receiving 10,000 IU of retinyl acetate per day for 25 days (Sudhakaran and Kurup, 1974) and in male Wistar rats receiving 60,000 IU a day for 8 days (Lacord-Bonneau *et al.*, 1972).

Barratt (1973) found that explants of pig articular tissue cultured in the presence of 5 or 10 IU of retinol per ml did not differ significantly from explants cultured in the absence of added retinol; however, if the explants included some marrow tissue, the presence of 5 or more international units of vitamin A per ml in the culture media resulted in extensive degradation of the cartilage.

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